

CLAIMS

1. A method for the production of polynucleotide molecules with modified properties, wherein at least one cycle comprising the following steps is completed:
 - (a) providing a population of single-stranded polynucleotide molecules, wherein the individual polynucleotides of said population have both homologous and heterologous sequence segments, and wherein in the population also strands are contained that are each completely or partially complementary to these single strands;
 - (b) formation of double-stranded polynucleotide molecules of the population of single-stranded polynucleotide molecules provided according to step (a) comprising double strands with different heterologous sequence segments;
 - (c) partial, exonucleolytic single-strand degradation of the double-stranded polynucleotide molecules produced according to step (b); and
 - (d) template-directed single-strand synthesis starting from the degraded ends of the partially degraded double strand produced according to step (c),wherein steps (c) and (d) may be carried out subsequently or contemporaneously.
2. The method according to claim 1, wherein more than one cycle comprising steps (a) to (d) is completed.
3. The method according to claim 2, wherein the degradation length of the exonucleolytic degradation, according to step (c) of the method of the invention is constantly reduced with increasing number of cycles.
4. The method according to any one of claims 1 to 3, wherein the regio selectivity of the combination of partially degraded and newly synthesized strands is regulated by the control of the partial, exonucleolytic single-strand degradation according to step (c).

5. The method according to ~~any one of claims 2 to 4~~, wherein after one, several or all cycles a selection step is carried out, and said selection step relates to either the genotype or the phenotype or to both the genotype and the phenotype of the polynucleotide.
6. The method according to ~~any one of claims 1 to 5~~, wherein the population of single-stranded polynucleotide molecules provided according to step (a) are polynucleotide molecules from the mutant distribution of a quasi-species.
7. The method according to ~~any one of claims 1 to 6~~, wherein the polynucleotide strand subjected to an exonucleolytic single-strand degradation and single-strand synthesis consists of DNA.
8. The method according to ~~any one of claims 1 to 7~~, wherein the exonucleolytic single-strand degradation of the double-stranded polynucleotides according to step (c) takes place in 3'-5' direction.
9. The method according to claim 8, wherein in step (c) exonuclease III from E. coli is used for the 3'-exonucleolytic single-strand degradation.
10. The method according to claim 8 or 9, wherein in step (c) exonuclease I from E. coli is used for the 3'-exonucleolytic single-strand degradation of unpaired segments of the heteroduplices.
11. The method according to ~~any one of claims 1 to 7~~, wherein the exonucleolytic single-strand degradation of the double-stranded polynucleotides according to step (c) takes place in 5'-3' direction.
12. The method according to claim 11, wherein in step (c) T7-exonuclease Gene 6 from the bacteriophage T7 is used for the 5'-exonucleolytic single-strand degradation of the double-stranded polynucleotides.
13. The method according to claim 11 or 12, wherein in step (c) exonuclease VII from E. coli is used for the 5'-exonucleolytic single-strand degradation of unpaired segments of the heteroduplices.
14. The method according to ~~any one of claims 1 to 13~~, wherein one of the two ends of the polynucleotide double-strand is modified in such a way that it is

protected from the 3'- or 5'-exonucleolytic single-strand degradation according to step (c).

15. The method according to claim 14, wherein the modification takes place by selective insertion of thioesters or by cleavage with a restriction enzyme leading to a 3'-overhang, or by first providing one of the two strands as circular single strand, or by covalent coupling with a compatible, circular polynucleotide molecule.
16. The method according to ~~any one of claims 1 to 15~~, wherein before the exonucleolytic single-strand degradation according to step (c), single-strand nicks are introduced into the double-stranded polynucleotide molecules.
17. The method according to claim 16, wherein on average one or less than one single-strand nick per double-stranded polynucleotide molecule is introduced.
18. The method according to claim 16 or 17, wherein single-strand nicks are introduced into the double-stranded polynucleotide molecules by means of sequence-specific nicking enzymes.
19. The method according to claim 16 or 17, wherein single-strand nicks are introduced into the double-stranded polynucleotide molecules by means of sequence-unspecific nicking enzymes.
20. The method according to ~~any one of claims 16 to 19~~, wherein the exonucleolytic single-strand degradation according to step (c) takes place both in 5'-3' direction and in 3'-5' direction.
21. The method according to claim 20, wherein Bal31-nuclease from the culture medium of *Alteromonas espejiana* Bal31 is used for the contemporaneous 5'- and 3'-exonucleolytic single-strand degradation in step (c).
22. The method according to ~~any one of claims 16 to 19~~, wherein the exonucleolytic single-strand degradation according to step (c) takes place by means of a polymerase with 5'-exonucleolytic activity.
23. The method according to ~~any one of claims 7 to 22~~, wherein the template strands in step (d) are DNA molecules and one or more DNA-dependent DNA polymerases are used for the template-directed single-strand synthesis.

24. The method according to claim 23, wherein polymerase I from E. coli is used.
25. The method according to claim 23, wherein one or several thermostable DNA polymerases are used.
26. The method according to claim 25, wherein Taq DNA polymerase from Thermus aquaticus, Tth DNA polymerase from Thermus thermophilus HB8 or Tfi DNA polymerase from Thermus flavus is used.
27. The method according to ~~any one of claims 16 to 26~~, wherein the 3'-ends of the newly synthesized segments are covalently coupled with the 5'-ends of the segments partially degraded in an exonucleolytic manner.
28. The method according to claim 27, wherein the covalent coupling takes place by means of T4 DNA ligase from the bacteriophage T4.
29. The method according to ~~any one of claims 7 to 22~~, wherein the template strands in step (d) are RNA molecules and one or more RNA-dependent DNA polymerases are used for the template-directed single-strand synthesis.
30. The method according to claim 29, wherein AMV reverse transcriptase from the avian myeloblastosis virus, HIV reverse transcriptase from the human immunodeficiency virus, M-MuLV-reverse transcriptase from the Moloney murine leukemia virus or Tth DNA polymerase from Thermus thermophilus with intrinsic reverse transcriptase activity are used.
31. The method according to ~~any one of claims 1 to 6~~, wherein the polynucleotide strand subjected to exonucleolytic single-strand degradation and single-strand synthesis consists of RNA.
32. A kit containing components and instructions for carrying out the method according to ~~any one of claims 1 to 31~~.